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## 2497 Fluoride -Treated Bone-Derived Material Promotes Osteoblast Proliferation and Enhances Collagen Expression *In vitro*.

C. G. FRONDOZA, R.Z. LEGEROS, V.L.TSEN, D.S.HUNGERFORD (Johns Hopkins University, Baltimore MD and New York University College of Dentistry, NY)

Earlier studies indicate that fluoride may retard bone resorption and may influence osteoclast function. Little is known about the response of osteoblasts to fluoride treated bone. The purpose of this study was to evaluate the effect of fluoride treated bovine bone derived materials on human osteoblasts. Methods: Particles of cortical bone were treated to remove the organic phase and subsequently (a) sintered at 950ºC; or (b) treated with 2% NaF and then sintered at 950ºC. The samples were washed for 4 d and irradiated by uv for 48 hrs. They were then incubated with either human osteoblasts-like MG63 cells or normal human osteoblasts ($10^5$cells/ml) at 30ºC, 5% $CO_2$ for 4 days. Proliferative capacity was determined by incorporation of $^3H$ thymidine into TCA precipitable DNA. Collagen expression was determined by RT-PCR with GAPDH as the housekeeping control gene. Cells incubated with culture media alone served as controls. Results: MG63 cells incubated with (a) F-treated and (b) untreated particles had DNA synthetic rate greater than that of cells in (c) control media alone (a = 3.8 ± 0.3, b 0.4, c = 1.5 ± 0.1 $10^6$ cpm/ ml respectively; P< 0.05). Collagen expression of normal osteoblasts was enhanced in the presence of F-treated particles compared to untreated and medium control. Conclusion: This study showed that human osteoblasts respond favorably to F treated bovine derived material. F-treated bovine derived mineral may serve as clinically useful bone substitute to repair bony defects.

*(Supported in part by research grant no. DE12388 from the National Institute of Dental Research of the National Institute of Health and the Good Samaritan Hospital Endowment Fund).*

## 2498 DNA Sequence Analysis of the *Fusobacterium nucleatum* plasmid, pFN1.

Susan Kinder Haake[*1] and Sydney M. Finegold[2]. UCLA School of Dentistry[1] and the Wadsworth Anaerobe Lab at the West Los Angeles VA Medical Center[2], Los Angeles, CA.

*Fusobacterium nucleatum* is part of the normal microbiota of human mucous membranes, and is often isolated from human infections. Putative virulence determinants have been identified, but their evaluation has been hampered by a lack of systems for genetic manipulation. We isolated plasmids from several strains of *F. nucleatum* for use in the development of gene transfer systems. Analysis of the DNA sequence of the plasmid pFN1, isolated from a clinical strain of *F. nucleatum*, is reported in this abstract. A preliminary restriction map was generated and a 1.5 kb *Hin*DIII fragment of pFN1 was cloned into pBluescript to generate pHS9. Both strands of pFN1 were sequenced in overlapping fragments by automated DNA sequencing using pHS9 and pFN1 DNA as templates. The DNA sequence was compiled using DNA Strider and Clustal V software. Homology searches were performed through the National Center for Biotechnology Information. Sequence analysis revealed that pFN1 consists of 5887 base pairs with 7 putative open reading frames. The predicted amino acid sequence of two open reading frames, ORF5 and ORF1, demonstrated significant regions of identity and similarity with previously described proteins. A 154 base pair region of ORF5 was found to have 27-28% identity and 48-49% similarity with previously identified plasmid replication proteins. One of the plasmids, pUCL287, is known to be a theta-replicating plasmid. The pFN1 DNA sequence upstream of ORF5 demonstrates six perfect 22-base pair repeats ("iterons"), preceded by an approximately 200 base pair A-T rich region. This organization is consistent with iteron-regulated theta-replicating plasmids. Significant amino acid identity and similarity was evident between ORF1 and relaxase proteins of several plasmids from Gram-positive species. The related regions correspond to 3 of 4 consensus sequences that have been defined for these proteins, which are involved in the initiation of conjugal transfer of plasmid DNA. These results suggest that pFN1 is a theta-replicating iteron-regulated plasmid which encodes a protein related to plasmid mobilization. This work was supported by grants from the UCLA School of Dentistry Opportunity Fund, the UCLA Academic Senate, and PHS Grant DE 12639.

## 2499 Acquisition of plasmin activity by *Fusobacterium nucleatum*: potential roles in tissue destruction.

H. Darenfed, D. Grenier* and D. Mayrand. (Groupe de Recherche en Écologie Buccale, Université Laval, Québec, CANADA).

*Fusobacterium nucleatum*, a Gram-negative anaerobic bacterium, has been associated with a variety of oral and non-oral infections such as periodontitis, pericarditis, bone infections and brain abscesses. Several studies have shown the role of plasmin, a plasma protease, in increasing the invasive capacity of microorganisms. In this study, we investigated the binding of human plasminogen to *F. nucleatum*, and its subsequent activation into plasmin. Bacterial cells were incubated with human plasminogen, and the binding was demonstrated by a dot-blot assay using an anti-plasminogen antibody. The binding activity was found to be heat resistant and to involve lysine residues present on the bacterial cell surface. The activation of plasminogen-coated cells was possible by incubation with either streptokinase, urokinase or a *Porphyromonas gingivalis* culture supernatant. In the case of the *P. gingivalis* culture supernatant, a cysteine protease appears to be involved in the activation. Plasmin-coated *F. nucleatum* were found to degrade tissue inhibitor of metalloproteinases 1, fibronectin and to a lesser extent laminin. This study suggests a possible role for plasminogen in promoting tissue destruction and invasion by non-proteolytic bacteria such as *F. nucleatum*.

This work was supported by FCAR, FRSQ, Fonds Émile-Beaulieu and Laboratoire de contrôle microbiologique.

## 2500 S-layer of *Campylobacter rectus*:role in gingival fibroblast (HGF) adherence and cytokine stimulation.

K. REDDI*, JL. EBERSOLE & SC. HOLT (University of Texas Health Science Center at San Antonio, 78284-7894, USA).

*Campylobacter rectus* (*C. rectus*) is an important member of the periodontopathic microbiota associated with inflammatory events responsible for alveolar bone resorption and tissue destruction. *C. rectus* express a proteinaceous surface layer (S-layer) external to its outer membrane which is thought to provide protection from the host during infection. This study investigated differences in the ability of *C. rectus* strain 33238 (S-layer present, *crs+*) and its spontaneous mutant (S-layer absent, *crs-*) to bind to HGF. HGF were incubated with radiolabeled *crs+* and *crs-* at Multiplicity of Infection's (MOI's) ranging from 16:1 to 10000:1 and binding to HGF determined. *Crs-* bound to HGF in a dose-dependent manner whereas *crs+* bound poorly suggesting that the presence of the S-layer inhibited the components necessary for binding of *C. rectus* to HGF. To investigate the factors required for binding, *C. rectus* were pretreated with proteinase K (0.5mg/ml; 1hr; 37ºC). SDS-PAGE analysis revealed that exposure to proteinase K resulted in complete digestion of the S-layer *Crs+* proteinase K treated cells bound 100% greater to HGFs than control untreated cells, supporting a function of the S-layer makes *C. rectus* invisible to this host cell. To determine whether *crs+* and *crs-* could stimulate the release of IL-1β, IL-6 and IL-8, HGF were exposed to *crs+* and *crs-* at MOI's from 2:1 to 1000:1. After an overnight incubation the supernatants were tested for cytokines by ELISA. *Crs+* and *crs-* stimulated the release of IL-6 and IL-8 in an equipotent and dose-dependent manner over the MOI range of 8:1 to 1000:1. No IL-1β release could be detected. However, RT-PCR revealed mRNA for IL-1β in HGF exposed to both *crs+* and *crs-*. Cytokine stimulation was inhibited by HGF exposed to *crs+* and *crs-* treated with 30 μg/ml of polymyxin-B, suggesting that lipopolysaccharide (LPS) is the component on *C. rectus* responsible for this activity. This study demonstrated that: (1) the presence of the S layer inhibits the binding of *C. rectus* to HGF; and, (2) that LPS is the component responsible for stimulating IL-1β, IL-6 and IL-8 production by HGF. Supported by DE-10960

## 2501 Interleukin Secretion by the GroEL-Like Protein from *Campylobacter rectus*.

D. HINODE*, S. TANABE, O. MIKI, K. MASUDA, M. YOSHIOKA and R. NAKAMURA (Department of Preventive Dentistry, School of Dentistry, The University of Tokushima, Japan).

Recently, considerable attention has been given to the potential role of bacterial heat shock proteins in the inflammation of host tissue. The aim of this study was to investigate the biological effects on the human gingival fibroblast (HGF) by the GroEL-like protein from *Campylobacter rectus*, a putative periodontal pathogen. The native GroEL-like protein was prepared from *C. rectus* cells by affinity chromatography on adenosine 5'-triphosphate-agarose followed by high performance liquid chromatography on Superose 6. Two hundred microliter of the sample (3.0 μg/ml GroEL-like protein) was added onto HGF confluent monolayer in each well of the 96-well plastic tray, and then incubated at 37°C for 22h in a $CO_2$ incubator. The supernatant from the HGF culture was collected and the IL-6 and the IL-8 content was measured using commercial assay kits. The assay for the quantification of HGF viability was also performed. In this study, no significant difference could be detected between the control (phosphate buffered saline) and the sample with respect to cell viability. However, the amounts of IL-6 and IL-8 were increased by 5.4- and 3.5-fold, respectively. These data indicate that the *C. rectus* GroEL-like protein is capable of enhancing IL-6 and IL-8 production in HGF and it could be a virulence factor in periodontal disease. This work was in part supported by the Ministry of Education, Science and Culture of Japan.

## 2502 Oral carriage of *Helicobacter pylori* in rural Guatemala:

SA DOWSETT[1*], L ARCHILA[2], KA VASTOLA[3], CG BONILLA[4], VA SEGRETO[3] and MJ KOWOLIK[1]. ([1]Indiana School of Dentistry, IN, [2]Faculta Odontologia UMG, Guatemala City, [3]The Procter and Gamble Company, Cincinnati, OH, [4]IMSS, Mexico City, [5]UTHSCSA, TX).

There is now overwhelming evidence to implicate *H. pylori* in the etiology of a spectrum of gastroduodenal diseases. Prevalence of infection is population-dependent but particularly high in non-industrialized countries where carriage may reach 100%. The route of infection is still unclear although there is evidence for oral-oral and feco-oral transmission. The aim of this study was to determine whether the oral cavity is a reservoir for *H. pylori*, in an isolated population of Central America. A full medical history was taken from 242 study participants (112 males, 130 females) with age range 12-75 years. A finger-prick blood sample was obtained for serology and *H. pylori* antibody status measured using an ELISA-based onsite serology kit, QuickVue® (Quidel). Periodontal pocket depths were measured at 6 sites per tooth and bacterial samples collected from oral sites using sterile absorbent points. Similarly, samples were taken from the nail bed of the index finger of the dominant hand. *H. pylori* was detected in samples by nested PCR, using previously described primers of the 16s rRNA genes (Ho *et al*). In subjects 12-17 yrs 40% were seropositive for *H. pylori* compared with 90% of those 55-64 yrs. At least 75% of oral sites were positive for *H. pylori* in 49% of subjects and 87% of subjects had at least one positive oral sample. There was no significant association with pocket depth. Positive nail samples were found in 58% of subjects. In conclusion, the prevalence of oral *H. pylori* in this population is higher than generally reported in the literature and suggests the oral cavity may be a significant reservoir for *H. pylori*. Detection of *H. pylori* under the finger nail also suggests that infection may occur via the feco-oral route. (Ho *et al* 1991. J Clin Microbiol 29: 2543-49).

## 2503 Colonization of Human Dental Plaque by Helicobacter Pylori.

A. A. KHAN* AND A. K. BUTT (Shaikh Zayed Hospital, Lahore, Pakistan).

Helicobacter pylori is now generally accepted to play a key role in acid related and neoplastic gastroduodenal diseases. Apart from the gastric antral mucosa, the organism has been recovered from the human dental plaque, which could serve as an important extra gastric sanctuary and a possible source of recrudescence in patients of peptic ulcer. Data on H. pylori colonization of dental plaque is very contradictory with high prevalence rates in Asian countries in contrast to low figures from Western countries. We present data on H. pylori colonization of dental plaque in 125 males and 53 females with a mean age of 36 ± 9 years. Six dental plaque specimens were obtained with a sickle probe; two were inoculated into CLO test gel (Delta West, Australia) and the remaining four were used to prepare cytology slides stained with Giemsa's stain. Chi-square test was used for statistical analysis; a p value ≤0.05 was considered significant. CLO test was positive on 100% of specimens. Cytology for H. pylori was positive in 173 (97%) cases. One hundred and forty three (80%) cases had heavy plaque deposits and all were positive on cytology. Two patients with minimum and 3 patients with moderate amount of plaque had negative cytology. Sixty six percent cases had a Community Periodontal Index of Treatment Needs score of 3 followed by a score of 4 in 17% and 5 in 9% while 4% each had scores of 1 and 2. No correlation was found between positive plaque cytology and severity of gingival or periodontal inflammation (p >0.05). The high prevalence of H. pylori colonization of dental plaque in this study could have important implications for epidemiology of H. pylori associated peptic ulcer disease.

## 2504 Detection of *Helicobacter pylori* in the stomach and the oral cavity of a Venezuelan population.

A. BERROTERAN, M TOMBAZZI, M CORRENTI, M CAVAZZA, R GONCALVEZ, M PERRONE. (Central University of Venezuela, MSAS, Caracas, Venezuela).

The presence of *H. pylori* in the stomach is strongly associated with chronic gastritis and ulcer disease and is a risk factor for gastric cancers. The microorganism may be transmitted orally and has been detected in dental plaque, saliva, and feces, but the hypothesis that oral microflora may be a permanent reservoir of this bacteria is still controversial. To evaluate the potential of the oral cavity in this process, the presence of *H. pylori* was determined in 26 patients from the Clinical Hospital University, Central University of Venezuela attending for routine gastroscopy. Gastric antrum biopsies were taken for CLO (rapid urease test) and culture. Supragingival plaque and saliva specimens (1-2 ml) were collected prior to undergoing endoscopy. All oral and gastric biopsy samples were cultures on selective and non selective media. Plates were incubated at 37° C for five days in a microaerobic atmosphere. Colonies resembling *H. pylori* on the agar were picked off for Gram staining, oxidase, catalase, and urease test. *H. pylori* was detected in antral samples in 61,5% (16/26) by CLO and 50% (13/26) were positives in cultures. Three of the samples positives by CLO were negative by culture 81,25% (13/16). The dental plaques samples and saliva specimens were positive for *H. pylori* in 15,3% (4/26) and 23% (6/26) respectively. All the patients positives in dental plaque culture were positive in saliva culture. Only one patient *H. pylori* culture positive in dental plaque and saliva was negative in the biopsy sample. We concluded that the oral cavity may be an important reservoir for *H. pylori* and the detection of this bacteria at various oral sites in patients with gastritis indicates that oral-spread is a potential route of transmission.

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